warranted to determine their role in risk stratification strategies.

Table. Patient characteristics

Variable	*No. (%) or mean (N = 697)*
Age group, years	
44-55	206 (30)
56-65	336 (48)
66-74	155 (22)
Current smoker	48 (7)
Diabetes mellitus	49 (7)
Hypertension	261 (38)
Hyperlipidemia	330 (48)
Peripheral vascular disease	32 (4.7)
Stroke	13 (1.9)
Angiographic CAD	22 (3.47)
Body mass index, kg/m^2	29.7

CAD, Coronary artery disease

Thrombospondin-1–Induced Vascular Smooth Muscle Cell Migration Is Functionally Dependent on MicroRNA-21

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Objective(s): Thrombospondin-1 (TSP-1) is a matricellular glycoprotein released from platelets at sites of arterial injury. After angioplasty, TSP-1 plays a role in the formation of the neointima. MicroRNAs (miR) are small noncoding RNAs that traditionally function by binding target gene messenger RNA and inhibiting protein translation. Previous study has shown that miR-21 is upregulated after angioplasty, and inhibition of miR-21 leads to decreased intimal hyperplasia. In this study, we examined the acute effects of miR-21 inhibition on vascular smooth muscle cells (VSMCs).

Methods: Quiescent VSMCs were treated for 20 minutes with 25 nM miR-21 inhibitor. Migration toward TSP-1 (20 μg/mL) or the negative control, serum-free media (SFM) was assessed using a modified Boyden microchemotaxis chamber. Quiescent VSMCs were acutely treated with the miR-21 inhibitor and then exposed to TSP-1 or basal media. Protein was collected, and phosphorylated extracellular signaling kinase (pERK) 1/2 expression was assessed by Western blot. Densitometry was converted to percent positive control. Quantitative real-time polymerase chain reaction (qRT-PCR) for TSP-1, hyaluronic acid synthase 2 (HAS2), and transforming growth factor-β2 (TGF-β2) was performed on samples acutely treated with miR-21 inhibitor before a 6-hour exposure to TSP-1. Statistical analysis was performed using analysis of variance with post hoc testing. Significance was set at $P < .05$. Comparisons for migration and Western blots were made to positive control.

Results: TSP-1 increased VSMC migration compared with serum-free media alone (100% ± 9.6% vs 43.7% ± 7.4%). miR-21 inhibitor treatment reduced VSMC migration to levels comparable to negative control (44.8% ± 4.9%). TSP-1 increased pERK 1/2 compared with basal media (p42: 100% ± 0% vs 78.6% ± 6.4%; p44: 100% ± 0% vs 76.2% ± 2.3%). miR-21 inhibition returned pERK to levels comparable with basal media (p42: 77% ± 6.2%; p44: 71% ± 9.8%). miR-21 inhibition had no effect on TSP-1–stimulated expression of TSP-1, HAS2, or TGF-β2 ($P > .05$).

Conclusions: Acute inhibition of miR-21 leads to a functional decrease in VSMC migration toward TSP-1. Additionally, acute inhibition of miR-21 decreased activated ERK 1/2, indicating a direct role for miR-21 in the TSP-1 signaling cascade. However, miR-21 does not acutely affect HAS2, TSP-1, or TGF-β2 gene expression. These data indicate that miR-21 may directly modulate cell functions and signaling pathways in ways other than by inhibition of protein translation.

Epigallocatechin-3-Gallate Is the Most Potent Phytochemical Inhibitor of Experimental Intimal Hyperplasia in the Murine Carotid Artery

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Objective(s): This study evaluated the effect on intimal hyperplasia of intraperitoneal (IP) administration of the phytochemicals resveratrol, allicin, sulforaphane (SFN) and epigallocatechin-3-gallate (ECGC) in the murine carotid artery injury model.

Methods: Myointimal hyperplasia (MIH) was induced in carotid arteries of 27 rats by introduction of a wire with a diameter near that of the artery (~0.2 mm.). The control group (n = 5) was injected with saline, whereas the experimental groups were injected daily with the corresponding phytochemical. Intima-to-media ratios (IMR) were quantified on 2-week specimens from photomicrographs using J software (National Institutes of Health, Bethesda, MD). Statistical significance was measured by the Student *t* test.

Results: IP injections of phytochemical suppressed intimal hyperplasia in rat carotid arteries at 2 weeks compared with controls and is summarized in the Table. Further studies performed in the EGCG group consisted of Ki-67 proliferation indices and analysis of extracellular signal-regulated kinase, c-Jun N-terminal kinases, and p-38 expression. Ki-67 index was significant reduced in EGCG animals (3.3 ± 1.0) compared with controls (6.2 ± 1.3). Extracellular signal-regulated kinase expression was similar suppressed in the EGCG group. No change were noted for c-Jun N-terminal kinases and p-38 expression.

Conclusion: Intraperitoneal injection of the phytochemical ECGC has significant suppressive effects on the development of myointimal hyperplasia in our carotid artery injury model. Other phytochemicals showed lesser effects.

Table.

Study group dose	*Dose (μg)*	*MIH (mm^2)*	*Reduction of control (%)*	P	*IMR*	*Reduction of control (%)*	P
Control		0.22 ± 0.03			0.70 ± 0.08		
Allicin	15	0.12 ± 0.02	44	<.05	0.41 ± 0.05	40	<.05
Resveratrol	18	0.09 ± 0.03	50	<.05	0.36 ± 0.09	49	<.05
Sulforaphane	0.48	0.09 ± 0.03	57	<.05	0.30 ± 0.09	57	<.01
EGCG	10	0.8 ± 0.03	63	<.05	0.20 ± 0.05	72	<.01

EGCG, Epigallocatechin-3-gallate; *IMR,* intima-to-media ratios; *MIH,* myointimal hyperplasia.

Vascular Endothelial Growth Factor-A Inhibits Venous Marker Expression and Stimulates Arterial Marker Expression in Adult Venous Endothelial Cells

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Objective(s): Vascular endothelial growth factor (VEGF) stimulates angiogenesis and determines arterial identity in embryonic endothelial cells (ECs). When vein grafts are placed into arterial circulation, VEGF expression transiently increases; yet, later time points find its expression to be ultimately decreased. As such, the role of VEGF in normal vein graft adaptation to the arterial environment is not clear, and its role in adult EC phenotypic regulation remains unknown. Accordingly, we examined the role of VEGF in modulating phenotypic changes in adult venous ECs.

Methods: Adult mouse lung endothelial cells (MLECs) were stimulated with 100 ng/mL VEGF-A_{165} for 0 to 24 hours. In some experiments, cells were pretreated for 1 hour with VEGF receptor 2 (R2) neutralization antibody (1 μg/mL) or VEGFR2 kinase inhibitor (100 nM) before VEGF-A stimulation. EphB4, Ephrin-B2, delta-like ligand 4 (Dll4), neuropilin-1, and neuropilin-2 expression were examined using real-time quantitative polymerase chain reaction.

Results: At baseline, adult MLECs display a venous phenotype and are EphB4-positive and Ephrin-B2-negative. VEGF treatment resulted in downregulation of the venous marker EphB4 in a time-dependent (n = 8, $P = .0003$) and dose-dependent (n = 3, $P < .05$) manner. VEGF treatment increased expression of Dll4 in a time-dependent manner (n = 8; $P < .0001$) but did not affect Ephrin-B2 (n = 8, $P > .05$). Neuropilin-1 and neuropilin-2 expression were transiently increased at 1 hour and decreased by 24 hours (n = 8; $P < .0001$). Pretreatment with VEGFR2 neutralizing antibody or VEGFR2 kinase inhibitor inhibited EphB4 downregulation and the early increased expression of neuropilin-2 but did not affect the upregulation of Dll4 (n = 3, $P = .3276$) nor the early increased expression of neuropilin-1.

Conclusions: In adult venous ECs, VEGF inhibits expression of venous marker EphB4 and stimulates expression of arterial marker Dll4. VEGF regulation of EphB4 is mediated by VEGFR2. Our results suggest that regulation of Dll4 is independent of VEGFR2 might be through an alternate pathway. The finding that arterial marker Ephrin-B2 is not increased by VEGF treatment is consistent with our prior work showing that vein graft adaptation results in loss of venous EphB4 identity without a gain of arterial Ephrin-B2 identity. Vein graft adaptation to the arterial environment may depend on the plasticity of adult ECs and their ability to integrate VEGF signaling pathways to properly modify vessel phenotype.